Experimental Biochemistry
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Lecture – 04
Practical Aspects of Making Buffer

Hello, my name is Soumya De; I am an assistant professor in Indian Institute of Technology, Kharagpur. So, today I am going to discuss about preparation of two buffers. In this first week, we are discussing some of the basic concepts of experimental biochemistry.

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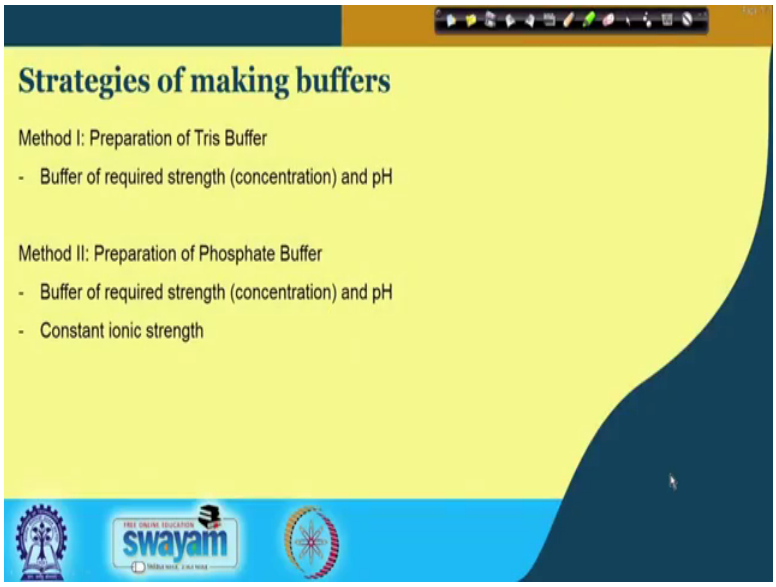

And I will discuss two methods of buffer preparation. And in the next few videos, you will actually see how we are going to make these buffers in the lab. So, there are two methods in the first method, we are going to prepare Tris buffer; and in the second method, we are going to prepare phosphate buffer. These two buffers have been discussed in the previous video lectures. So, in the first method, we are going to simply weigh out the Tris powder, dissolve it in water and adjust each it to the required pH, in this example, we are going to aim for a pH of 8.2.

So, one advantage of this method is it is a very straightforward method; you just have to weigh out the powdered buffer. In the second method, this is little bit more complicated. So, here we are going to prepare two solutions. So, we are going to take two different

phosphate salts. We are going to make two different solutions of those phosphate salts, and then we will meek mix the two solutions and get the required pH.

So, the difference I should point it out, and then I will again emphasize the differences as we go along. In the first one, what we are going to do is we are going to make the concentrated buffer solution so something that we refer to in the lab as a stock solution. So, even though let us say we want to make a buffer of 100 millimolar strength, the stock solution will be 10 times stronger. So, we will make 1000 millimolar or 1 molar concentrated sol buffer solution with the required amount of pH at the required amount of pH. And so in this method you have the pH as constant. So, if you want to make the buffer at pH 8.2, it will be at 8.2, but the concentration will be higher. So, if you need 100 millimolar, you dilute it 10 times. If you need 50 millimolar, you dilute it 20 times and so on and so forth. So, the concentration is variable ok.

In the second method, we will make two solutions of phosphate buffers, you will see which two solutions. Here, one solution will have low pH. So, we will see the lower pH solution will be at around 4.5. The higher pH solution will be at pH 9. So, any pH that you want you can get by mixing these two solutions in a particular ratio. So, if you want to get a solution of pH 6, you mix them in a particular ratio. If you want to get the final pH of pH 8, you will mix them in a different ratio.

So, in this second method, the pH is variable. In the first method the pH was fixed; the concentration was variable. In the second method the concentration of the phosphate ion is constant, but the pH is variable. So, here you can mix the two solutions which will not change the concentration of the phosphate ion, but you can get different solutions with different pH. So, one advantage of this method is that the ionic strength which is basically a measure of the concentration of all the ions in the solution. The ionic strength is maintained as a constant which is a disadvantage of the first method where we do not know the concentration of the ions as you will see because we (Refer Time: 04:10) it in some acid or base to get the required pH.

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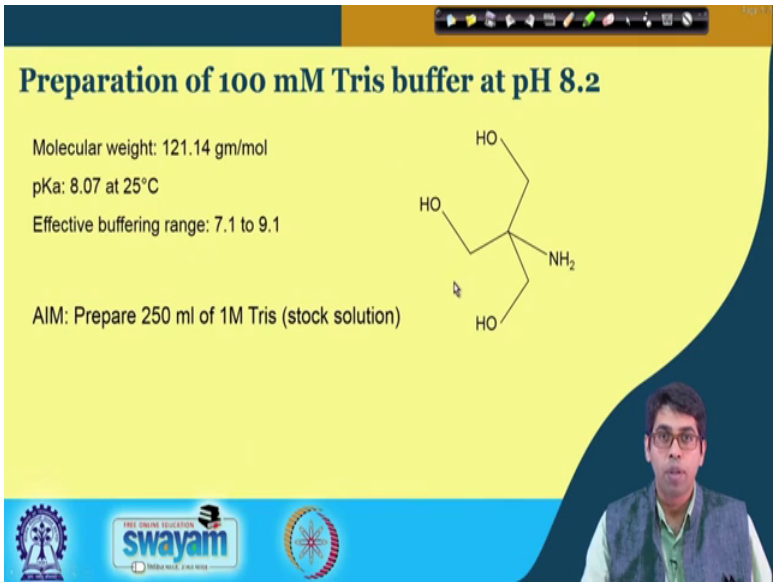

So, let us see how to make Tris buffer ok. So, as discussed previously this is the chemical structure of Tris. So, our aim is to prepare a 100 millimolar Tris buffer at pH 8.2. The molecular weight of Tris is 121.14 grams per mol. So, again these are some of the simple things that you always follow in the lab. You should always check the molecular weight written on the bottle or the box that from where you are taking the chemical ok. Because in the next example you will see that the molecular weight can change depending on the exact chemical that you are going to use. If you are going to use Tris, it should be 121.14 grams per mol. Its pKa is around 8 and which means that the effective buffering range of this buffer is between seven to one, 7.1 to 9.1 pH.

So, our aim is to make a stock solution which we will dilute subsequently to get this solution. So, our stock solution will be 10 times of this, so this is 100 millimolar, our stock solution will be 1 molar. And we are going to prepare a small volume. So, we will prepare 250 ml of 1 molar Tris.

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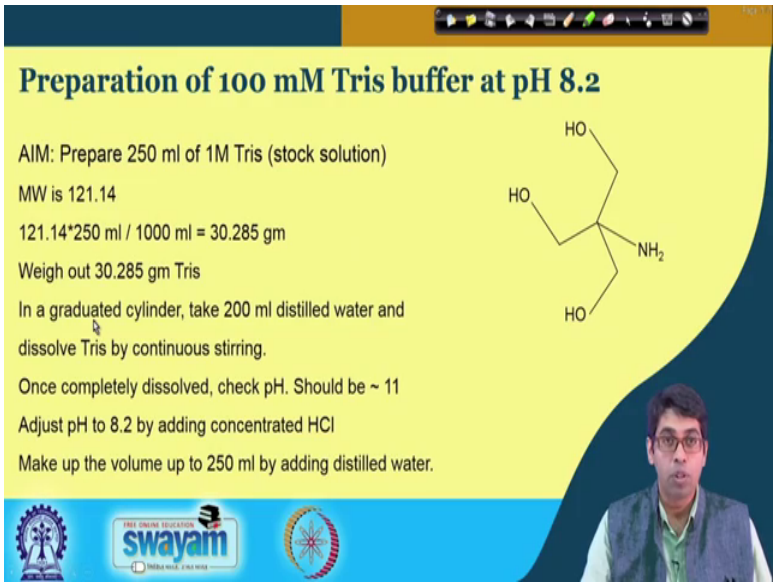

Since the molecular weight is known, you have to weigh out the required amount. So, if you weigh out this much grams that will be enough to make 1 liter of 1 molar Tris but we are not going to make 1 liter, we are going to make 250 ml. So, you multiply this with 250 ml and divide it by 1000 ml which is basically one-fourth, one-fourth of this. So, you get 30.285 grams. So, now, you weigh out 30.2 or 30.3 grams of Tris dissolve it in water. So, you should know the exact amount of water that you are going to use. In this case, we are going to use distilled water. So, you should take a graduated cylinder or a graduated beaker take 200 ml of distilled water in that and add your powdered Tris in that, dissolve it by continuous stirring.

Once it is completely dissolved, so you will know it is dissolved because even the water the solution will be completely clear ok. So, at this point, if you use a pH meter and check the pH, the pH of a solution will be somewhere close to 11, which means that it is much higher than what we are aiming for. So, we have to lower the pH which means that we have to add some sort of acid. Typically we use hydrochloric acid ok. So, we will use concentrated HCl, and add it drop wise to this solution.

While we are doing that we will also monitor the pH using a pH meter. You will see exactly how it is done in the lab. So, you keep on adding drop wise the hydrochloric acid and monitor the pH, and stop when the pH has reached 8.2. So, you have to do it very slowly because we will have to let the solution equilibrium, and the reading in the pH

meter also needs to be stabilized. Once you reach pH 8.2, the volume will be somewhere a little higher than 200 ml that you have started with ok. So, now you add water to make up the volume up to 250 ml. So, at this point you have 250 ml of 1 molar Tris which is your stock solution. We still do not have our required solution ok.

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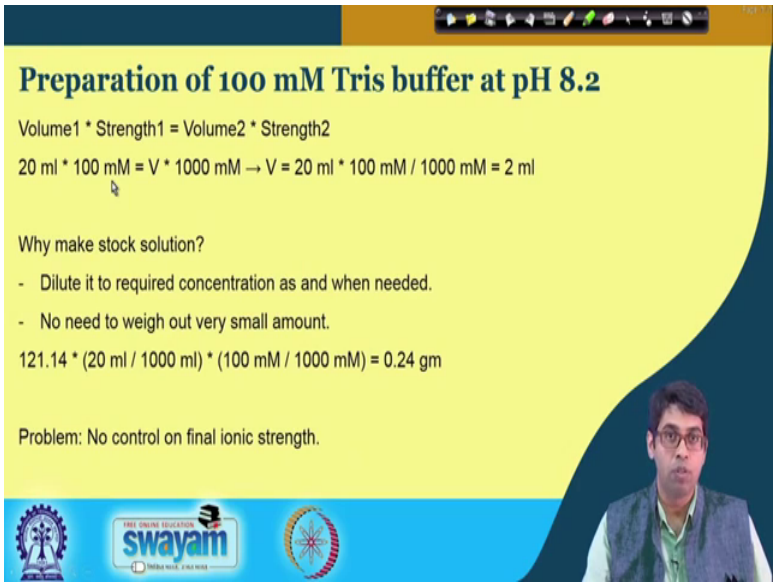

So, let us say for your particular experiment, you need only 20 ml of 100 millimolar Tris solution. So, to get that, we will use this formula, so this is something that is very useful and we will keep on using it on a almost on a daily basis in the lab. So, volume 1 multiplied by strength 1 equals to volume 2 multiplied by strength 2. So, in this case, this is the solution that we want. So, the volume that we want is 20 milliliter, and the strength or the concentrating of that solution that we want is 100 millimolar. So, this is our stock. So, we know the concentration of our stock which is 1000 millimolar, 1 molar is 1000 millimolar. So, we do not know what volume we have to take of the stock solution. So, we can solve for this unknown volume V, and it comes out to be 2 milliliter.

So, you take 2 milliliter of your 1 molar Tris solution, which is at a pH 8.2, you add 18 ml of distilled water ok, so that you get a final volume of 20 ml. And now you have 20 ml of 100 millimolar Tris which is at a pH of 8.2 you are adding a water so the pH will not change, it will remain at 8.2.

The advantage of this method is that once you prepare the stock solution you can dilute it to any concentration that you need. The other advantage is that you do not have to weigh

out a very small amount. For example, suppose if you want to make this 20 ml of 100 millimolar Tris directly by weighing out Tris and dissolve it dissolving it in water right. So, in this case, we will have to weigh out 0.24 grams of Tris. Sometimes your balance is the weighing balance in the lab are not accurate enough to be precise up to two decimal or three decimal point ok. So, in that case, you will introduce error.

And then, the other problem is that, you will dissolve it in water, the pH will be high, then you will have to add acid and it can you can overshoot your pH. So, if you add a little bit of more acid, the pH will go down and then you will have to add base and so on and so forth ok. So, it is much easier to work with larger volumes and larger amounts of salt, so that you can make a stock solution. And once you have that stock solution, you can dilute it and get whichever volume you need at whichever strength you need ok.

The biggest problem of this method is that you do not have any control on the final ionic strength because we are adding hydro concentrated hydrochloric acid. So, we do not know exactly how much of chloride ions we are introducing into the buffer. We know the concentration of Tris, but we do not know the concentration of the chloride ion. So, the ionic strength of your solution is a unknown in this type in this particular method, which is solved in the second method, because in some experiments it is important to know or have a very good estimate of the ionic strength of your solution.

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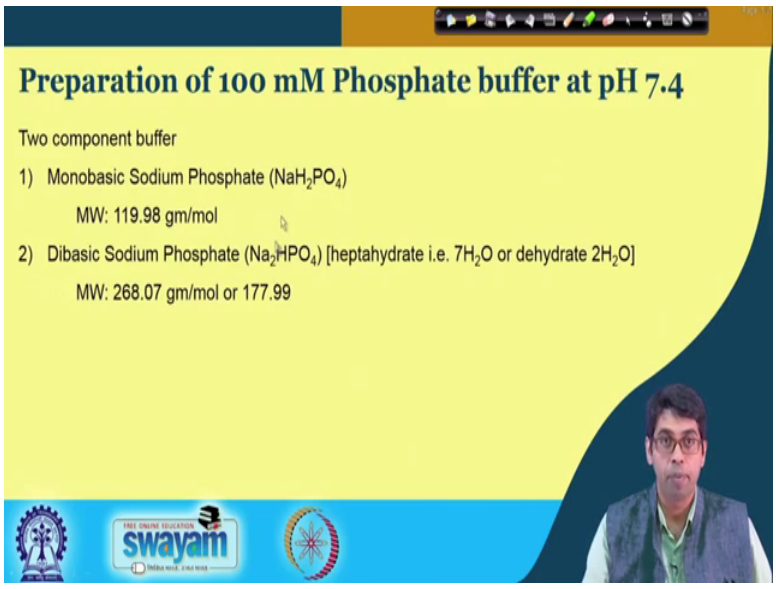

So, in the second method we want to prepare phosphate buffer ok. Again it is a 100 millimolar phosphate buffer. And we want to aim for a pH of 7.4. As I mentioned before it is a two component system. So, we are going to make two solutions and then we will mix them in a particular ratio. The first solution will be of monobasic sodium phosphate ok. So, you see Na H 2 PO 4. So, only one sodium ion, so that is why it is a monobasic and the other one is dibasic sodium phosphate Na 2 H PO 4.

Monobasic sodium phosphate has a molecular weight of almost 120 grams per mol. But when it comes to dibasic sodium phosphate, you have to be careful that is why I mentioned in a few minutes back that you should always check the bottle and look at the molecular weight of the compound that you are using. Dibasic sodium phosphate comes in two different forms one case heptahydrate which means that this has additional 7 molecules of water, and the other one is dihydrate so not dehydrate, it is will be dihydrate which means that this will have 2 water molecules ok.

So, now since the water molecules are there and different amounts of water molecules are there, the overall molecular weight will change. So, dibasic sodium phosphate heptahydrate has a molecular weight of 268 grams per mol, whereas disodium phosphate dihydrate has molecular weight of almost 178 grams per mol. So, depending on which one you are using you have to use that molecular weight and do the calculations accordingly.

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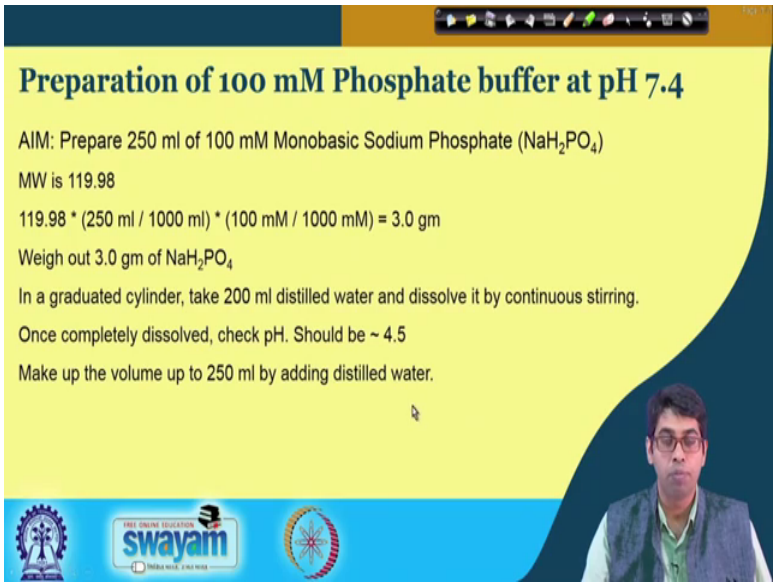

So, the first part is to prepare a 250 ml of 100 millimolar monobasic sodium phosphate. So, we are going to make monobasic sodium phosphate. We know the molecular weight. Since it is 250 ml, we are going to multiply the molecular weight by with 250 divided by 1000. And since we are not making a one molar solution, we are making 100 millimolar solutions, we have to multiply also with 100 millimolar divided by 1000 millimolar. So, the overall amount that we get is approximately 3 grams. So, you weight out 3 grams of monobasic sodium phosphate.

And just like before, you take a graduated cylinder or a graduated beaker, take 200 ml of distilled water. Dissolve this 3 grams of monobasic sodium phosphate with continuous stirring and then make up the volume up to 250 ml. If you check the pH of this solution, so remember we have not adjusted the pH if you check the pH of this solution it should be between 4 to 4.5 ok. So, it will be somewhere close to 4.5 pH. We are not going to adjust the pH of this solution. So, we know exactly how much of phosphate ion we have added in this solution.

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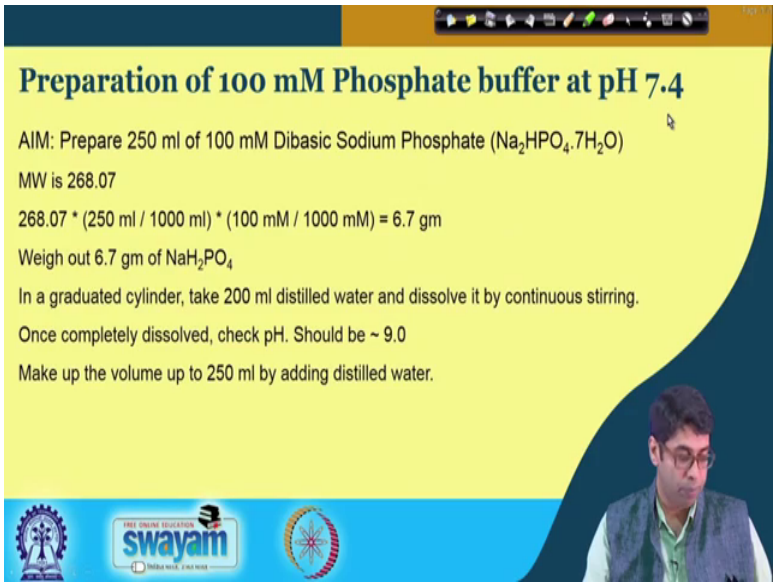

Similarly, we will make the second solution which is 100 millimolar of dibasic sodium phosphate 250 ml. So, let us say in the lab we have the heptahydrate form. So, then the molecular weight is 268 grams per mol. So, we will take so 268 grams per mol, and again multiply it by 250 divided by 1000, and multiply by 100 millimolar divided by 1000 millimolar. So, you get a number of this 6.7 grams. So, again weigh out 6.7 grams,

dissolve it in water, and make the volume up to 250 ml with distilled water. If you check the pH of this solution, it should be close to 9. So, the first one was around pH 4.5; the second one is around pH 9. We have not adjusted the pH of both solutions. So, we know exactly the amount of phosphate ion in both buffers. Now, we want to make 100 millimolar of phosphate buffer at pH 7.4.

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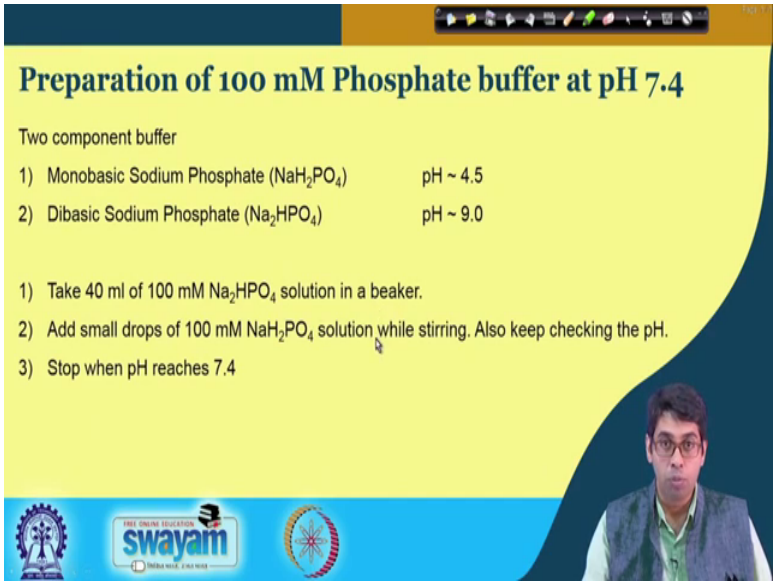

So, these are the two components. They are at these two different pH values. We want to get that at pH of 7.4. So, what we do is let us say we take 40 ml of the second solution which is dibasic sodium phosphate ok. Remember it is a pH 9 ok, 7.4 is close to pH 9, so that is why we have taken this solution. So, you have that in buffer you put a you put the pH probe in that, put a stirrer bar, start stirring, so that there is proper mixing and you monitor the pH. And using a dropper you can add drop wise the first solution which is at the lower pH.

So, what you will see is that the pH will start dropping. So, it starts at pH 9, and it will slowly go down. If you do this very slowly, then the system will be always at equilibrium, and you will know exactly what is the pH of your solution. So, when you reach at pH of 7.4, you stop. So, now, you have a solution of phosphate buffer, in both cases the concentration of phosphate is 100 millimolar. So, in this final solution also the concentration of phosphate is 100 millimolar, and the pH is now adjusted to 7.4. So, we

have try to get one solution with another solution, we have not used any concentration acid on base, we know exactly the amount of phosphate ion in the final solution.

So, in this method, we know the concentration of our ions, but you do not know exactly the volume which is not a big problem because you can use whatever volume you need to do your experiment ok. So, in the second method, we know the concentration of the ion and we can reach the required volume required pH by mixing these two solutions in a particular ratio. So, if you want to make a buffer solution with pH 6, you start with monobasic sodium phosphate and tight it in the dibasic solution, so that and again monitor it with a pH meter, so that you reach a pH 6. So, these experiments will be shown to you in the lab by our PhD students.

Thank you.